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Bone quality at the implant site after reconstruction of a local defect of the maxillary anterior ridge with chin bone or deproteinised cancellous bovine bone

Clinical Paper
Pre-Implant Surgery

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Abstract. The purpose of this study was to investigate the quality of bone at grafted implant sites in the anterior maxilla. Grafting of these sites was necessary because of insufficient bone volume in a buccopalatal direction (width at the top of the crest 1–3 mm).

Reconstruction was performed with chin bone ($N = 5$), chin bone and a resorbable Bio-Gide[®] GBR membrane ($N = 5$) or Bio-Oss[®] spongiosa granules in combination with a Bio-Gide[®] GBR membrane ($N = 5$). Biopsies were taken prior to implantation, i.e. 3 months after grafting with chin bone, and 6 months after grafting with Bio-Oss[®]. Evaluation was done by assessing the histological and histomorphometric characteristics of full-length biopsies taken from the actual implant site.

Both areas with non-vital bone and areas with apposition of bone and remodelling phenomena were observed in the chin bone group at the time of placement of the implants. Similar results were observed at implant sites reconstructed with a chin bone graft covered by a membrane. In the chin bone group without and with a GBR membrane, the mean total bone volume (TBV) was $55.2 \pm 6.8\%$ and $57.7 \pm 11.5\%$, respectively; the marrow connective tissue volume (MCTV) was $44.8 \pm 6.8\%$ and $42.3 \pm 11.5\%$, respectively. Remnants of the resorbable GBR membrane were not detected. In the Bio-Oss[®] group, at implant placement some newly formed bone was observed in the connective tissue surrounding the Bio-Oss[®] particles (mean TBV (newly formed bone) $17.6 \pm 14.5\%$), but most particles were surrounded by connective tissue. No convincing signs of remodelling were observed (mean remaining Bio-Oss[®] volume $40.5 \pm 9.3\%$; mean MCTV $41.9 \pm 13.1\%$). No implants were lost during follow up (12 months).

At the time of placement of the implants the grafting material (either chin bone or Bio-Oss[®]) is still not fully replaced by new vital bone. In case of Bio-Oss[®], most of the grafting material is even still present. Despite these differences, the 1-year clinical results were very good and comparable between the various grafting techniques applied.

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Reliable rehabilitation of the alveolar ridge with endosseous implants requires proper quality and quantity of alveolar bone at the implant site in order to achieve a good long-term prognosis^{3,11,33}. There is a diversity of surgical techniques and augmentation materials available for improving ridge conditions that meet these requirements^{4,13,14,16,17,24,25,30,32}. Such techniques include the use of grafting materials, either alone or in combination with barrier membranes^{5,6,15}. The most widely applied grafting material is autogenous bone, which has proven its value as grafting material for reconstruction of bony defects^{13,25,30}. Autogenous bone is harvested from both intra-oral and extra-oral sites. Autogenous bone is considered the "gold standard", but harvesting of autogenous bone requires surgery at a donor site. This among others results in increased morbidity, operation time and costs^{18,25,31}. These considerations have led to a search for bone substitutes that are biocompatible, non-infectious, non-antigenic and resorbable.

Reports on ridge augmentation are merely clinical studies on implant survival as a function of the augmentation material used and as a function of time^{8,36,37}. These clinical data are not indicative for the quality of the bone at the implantation site in case of reconstruction of local defects³. Histological analysis of the tissue at the implant site is needed focusing on a detailed characterisation of bone healing and remodelling. Several studies are available focusing on descriptive histology and histomorphometric analysis of autologous bone and bone substitutes. Resorption of autologous bone grafts (up to 56% of cortical bone grafts in 4 months) is reported in both animal and human studies^{3,23,27,37}. Conflicting results have been reported regarding the long-term behaviour of Bio-Oss[®]; some authors have described signs of resorption while others have reported a lack of breakdown^{1,2,14,19,23,28,34,35,38}. Histomorphometric analysis of biopsies taken 4–6 months after augmentation show a total bone volume (TBV) in autologous bone augmented sites of 37–47%^{1,3,12}. In Bio-Oss[®] augmented sites a TBV of 14–42% newly formed bone is reported after 6 months and a proportion of residual Bio-Oss[®] material of 13–30%^{1,3,12,38}.

A major drawback of most of the reports available in literature to date is that the biopsies taken for analysis are not derived from the actual implant site. The biopsies are harvested next to the implant site or perpendicular to the long

axis of the implant^{12,22,34,35}. This does not provide exact information on the quality of bone at the actual implant site. The biopsy can show the presence of autogenous bone or a substitute at the biopsy spot while the opposite might be true for the implantation spot. Therefore, the aim of this study was to investigate the quality of the bone at the implant site by assessing histological and histomorphometric characteristics of biopsies taken from the actual implant site and over the full length of the implant section in the anterior maxilla just prior to implantation. Additionally, clinical assessments were performed to compare the histological and histomorphometric findings with the clinical characteristics of the peri-implant gingiva.

Materials and methods

Fifteen patients, seven men and eight women with a mean age of 32.9 years (range 18–50 years), received an augmentation procedure for reconstruction of local defect of the anterior maxilla to provide a basis for reliable insertion of endosseous dental implants. All patients were non-smoking, partially edentulous and presented with a single tooth gap (Table 1). In all cases, the implantation site had to be reconstructed because of insufficient bone volume in a buccopalatal direction.

The defects were located in the "aesthetic zone" of the anterior maxilla. The pre-surgical evaluation disclosed an anatomy of the local bone responding to a class IV and V according to CAWOOD & HOWELL⁷, which did not allow the placement of an endosseous implant with sufficient initial stability. To reconstruct these defects, three treatment modalities were

applied: chin bone ($N = 5$), chin bone in combination with a Bio-Gide[®] GBR-membrane (Geistlich, Wolhusen, Switzerland; $N = 5$) and Bio-Oss[®] spongiosa granules (0.25–1.0 mm, Geistlich, Wolhusen, Switzerland) in combination with a Bio-Gide[®] GBR membrane ($N = 5$) (Fig. 1). A computer software program randomly placed the participating patients into these groups. Informed consent was obtained from all patients.

Surgical procedures

All patients were treated under local anaesthesia. Antibiotic prophylaxis was given for 72 h (amoxycillin 500 mg + clavulanic acid 125 mg (Augmentin[®], SmithKline Beecham, Zeist, The Netherlands), 1 h preoperatively and every 8 h postoperatively).

First a buccal pedunculated and to the buccal side reflected mucoperiosteal full-thickness flap was raised. From the top of the crest the incisions diverged to the buccolabial fold and were placed in such a way that the mucoperiosteal flap is on each side 5 mm wider than the area to be augmented. The interdental papillae were included in the flap. The extension of the incision to palatal is ± 5 mm from the top of the crest.

After mucoperiosteal reflection the orofacial bone width at the implant site was measured to the nearest quarter of a millimetre using a calliper. The width ranged from 1 to 3 mm. The cortical bone on the receptor site was perforated with a small round bur in order to create a bleeding bone surface and to open the cancellous bone.

Monocortical chin bone grafts ($N = 10$ patients) were harvested using a bur and chisels and fixed on the perforated recep-

Table 1. Personal data and local status of all 15 patients treated for ridge enlargement

Patient	Sex	Age	Site	Increase in width at the top of ridge (mm)	Augmentation material	GBR membrane
1	F	37	11	4	Chin bone	No
2	F	44	11	4	Chin bone	No
3	M	24	21	3	Chin bone	No
4	M	27	21	2	Chin bone	No
5	F	38	12	2	Chin bone	No
6	M	38	11	4	Chin bone	Yes
7	F	37	21	3	Chin bone	Yes
8	M	38	21	2	Chin bone	Yes
9	F	19	11	3	Chin bone	Yes
10	F	37	21	5	Chin bone	Yes
11	M	51	14	3	Bio-Oss	Yes
12	F	29	11	2	Bio-Oss	Yes
13	F	27	11	3	Bio-Oss	Yes
14	M	50	12	2	Bio-Oss	Yes
15	M	26	11	2	Bio-Oss	Yes

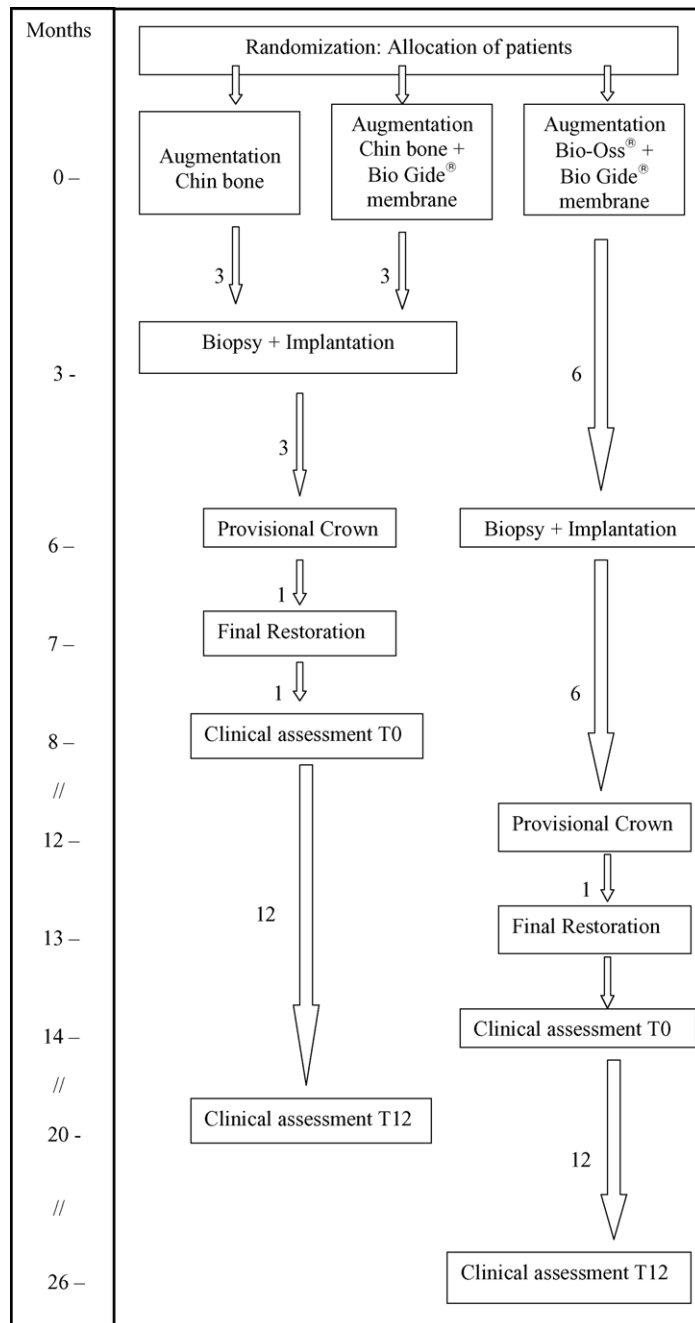


Fig. 1. Design of the study.

tor site (cortical side to the buccal) with a 1.5 mm titanium screw (Martin, Tutlingen, Germany)³⁰. Particulated chin bone was placed around the fixed block graft. In five patients, the chin bone graft was covered by a Bio-Gide® GBR membrane. The membrane was styled with a 3 mm extension over the bone margins of the defect and fixed with sutures (Vicryl 4-0, Ethicon, Johnson & Johnson, Amersfoort, The Netherlands).

Bio-Oss® granules ($N = 5$ patients) were mixed with blood derived from the operation site and placed on the perforated

cortical bone of the receptor site. A Bio-Gide® GBR membrane was applied to cover the grafts. The membrane was styled with a 3 mm extension over the bone margins of the defect and fixed with sutures (Vicryl 4-0).

Three months after augmentation of the anterior defect in the maxilla with chin bone or 6 months after augmentation with Bio-Oss®, the implants were placed. Again, the orofacial bone width at the implant site was measured to the nearest quarter of a millimetre. The screws used to fix the bonegrafts were removed. Subse-

quently a biopsy was taken from the implant site using a trephine bur (\varnothing 2.0 mm). Finally, after widening the biopsy spot to the required dimension using standard burs, ITI-Esthetic^{Plus} dental implants (Institut Straumann AG, Waldenburg, Switzerland) were placed. The implants were uncovered 6 months after placement.

A single operator (GMR) performed all surgical procedures.

At the day of uncovering the implants a temporary crown was placed, followed by the placement of the final crown 1 month later.

Clinical assessments

Clinical assessments were objectively performed at 1 (T0) and 12 months (T12) after placement of the final crown using the Gingiva Index (GI), pocket probing depth (PPD), and measuring the width of the attached mucosa (WAM) according to established methods in the literature (see below). Additionally, the level of the marginal buccal gingiva (MBGL) was measured. All clinical assessments were performed by a single investigator (L.M.) (Fig. 1).

1. LÖE & SILNESS Gingiva Index^{20,21}
0 = normal gingiva/mucosa around the tooth;
1 = mild inflammation; slight change in colour, slight oedema;
2 = moderate inflammation; redness, oedema and glazing;
3 = severe inflammation; marked redness and oedema, ulceration.

2. Pocket probing depth²⁹
Using a Merrit-B perioprobe the depth of the pocket on the buccal side of the implant-supported crown was measured. The distance between the marginal border of the gingiva and the tip of the pocket probe was scored as the PPD.

3. Width of attached mucosa⁹
The width of the attached mucosa buccal of the implant-supported crown was measured using the "Attached mucosa index"
0 = no keratinised epithelium is available;
1 = 1 mm or less keratinised epithelium;
2 = 1 or 2 mm keratinised epithelium;
3 = more than 2 mm keratinised epithelium.

4. Level of the buccal marginal gingiva
The level of the buccal marginal gingiva was scored by measuring the

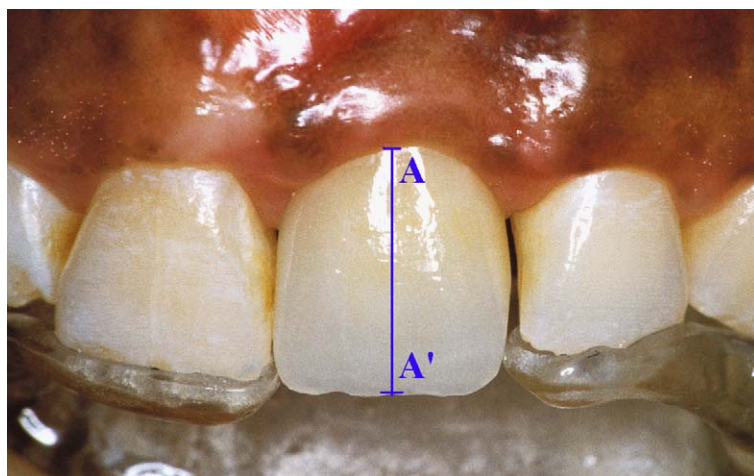


Fig. 2. The level of the buccal marginal gingiva was scored in the midline of the crown by measuring the distance between the marginal border of the buccal gingiva and the incisal edge of the implant supported crown.

distance between the marginal border of the buccal gingiva and the incisal edge of the implant supported crown (Fig. 2).

Histology

Biopsies were taken using a 2.0 mm trephine bur resulting in specimen with a core diameter of 1.7 mm. The biopsies were taken at the same location as the "implant-to-be-placed", at a similar angulation as the implant and up to a depth of 12 mm. By choosing a trephine bur with a diameter of 2.0 mm, the procedure of taking the biopsy did not interfere with the positioning of the implant (diameter 4.1 mm). Bio-Oss[®] specimens were easier to remove from the trephine than chin bone biopsies. All specimens could be removed in toto for evaluation.

The specimens were fixed in buffered formalin solution (4%). For histologic processing the specimens were washed in 0.185 M sodium cacodylate buffer for 2 h, dehydrated in ethanol and embedded in light-cured composite material (Technovit 7200 VLC, Kulzer, Frierichsdorf, Germany). Undemineralised longitudinal sections were cut from the central parts of the biopsies and ground to a thickness of 30 µm according to the method of DONATH & BREUNER¹⁰. Subsequently, the sections were surface stained with Toluidine blue/Pyronine G and evaluated histologically and histometrically.

Histological assessments

The biopsies were evaluated with regard to the vitality of the bone (presence of osteocytes in the osteocyte lacunae), signs of

remodelling (presence of osteoblast, osteoid and osteoclasts) and maturity of the bone (woven bone versus lamellar bone). Quantitative evaluation was performed with the aid of a light microscope and the use of ImageAccess (Imagic, Glattbrugg, Switzerland) software. As a region of interest (ROI) it was decided to analyse the superior two-thirds of the specimen in order to ensure to predominantly analyse grafted material.

For the bone histomorphometric analysis the following values were measured:

1. The *total bone volume* (TBV): the percentage of the section consisting of bone tissue.
2. The *marrow connective tissue volume* (MCTV): the percentage of the section consisting of marrow and connective tissue.
3. The *remaining Bio-Oss[®] volume* (RBOV): the percentage of the section consisting of Bio-Oss[®] material.
4. The *total mineralised mass* (TMM): the percentage of the section consisting of mineralised material (augmentation material (chin bone/Bio-Oss[®])/newly formed bone)

All histological assessments were performed by a single investigator (PS) not being involved in patient treatment.

Results

None of the 15 patients complained of significant pain either at the donor site or at the reconstructed local defect site in the maxilla. Similarly, no objective signs of infection were observed. There

was sufficient bone to place implants with a length of 12 mm in all cases. The width of the orofacial bone at the implant site gained 2–5 mm (Table 1). Clinically, when compared to the implant sites reconstructed with chin bone grafts, the bone at the implant site reconstructed with Bio-Oss[®] was not as compact as the bone at the sites reconstructed with chin bone. Nevertheless, the initial stability of the implants was good in all cases. No implants were lost during the follow up.

Clinical assessments

At T0, five implants showed mild inflammation (Gingiva Index Score 1), while the other 10 implants had normal peri-implant tissue (Score 0). Twelve months after placement of the final crown (T12), one implant showed mild inflammation and 14 implants had a normal peri-implant gingiva/mucosa. The mean PPD on the buccal side of the implant-supported crown was 3.0 ± 1.4 mm at T0 and 3.3 ± 1.8 mm at T12. All cases showed an attached mucosa of 2 mm or more around the implant supported crowns. The distance between the marginal border of the buccal gingiva and the incisal edge of the implant supported crown decreased with 0.24 ± 1.04 mm between T0 and T12.

Histological assessments

Mineralised material with a trabecular bone pattern was found in all biopsies derived from the augmented areas with some trabecular bone present in the biopsies derived from areas grafted with Bio-Oss[®], and both trabecular and compact bone from areas grafted with chin bone. In all groups, a mixture of mature and immature bone was present.

In specimen taken from areas grafted with chin bone, 3 months after grafting, the grafted particles were (partly) surrounded by layers of newly formed bone (Fig. 3A). All 10 biopsies from areas grafted with chin bone contained such areas. Part of these particles showed empty lacunae suggesting that these particles were non-vital, while other particles or parts of them clearly showed lacunae occupied by osteocytes (Fig. 3B). All chin bone biopsies showed signs of remodelling as shown by the presence of osteoblasts, apposition of osteoid and resorption lacunae occupied with multinuclear osteoclasts. Apposition of bone and remodelling phenomena were observed on both non-vital and vital chin bone particles. No differences

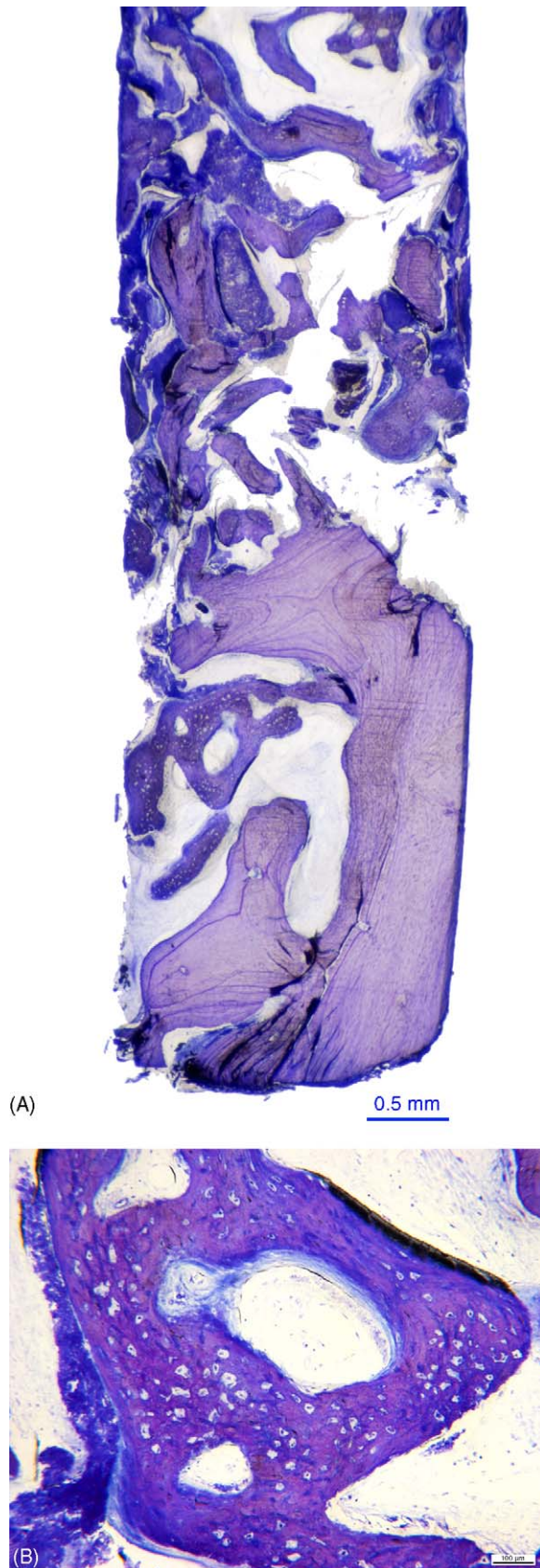


Fig. 3. (A) Section of a biopsy specimen after augmentation with chin bone. The biopsy is composed of larger and smaller cortical bone particles. Newly formed bone is clearly visible. (B) Detail showing vital, newly formed bone. In most lacunae osteocytes are present.

were observed between biopsies derived from grafted areas that were covered by a resorbable membrane or not. Remnants of the Bio-Gide[®] membrane were not detected.

In specimens taken from the Bio-Oss[®] augmented sites, 6 months after grafting, some newly formed bone was present in the connective tissue surrounding the Bio-Oss[®] particles, but most of the particles were surrounded by connective tissue (Fig. 4A and B). Bio-Oss[®] particles were present in four out of five biopsies. In the biopsies with Bio-Oss[®] particles neither signs of inflammation nor signs of foreign body reaction were seen around the Bio-Oss[®] particles. A minority of the particles was in contact with newly formed vital bone with lacunae occupied by osteocytes. Thus, at least locally, there seems to be some bone-apposition on the surface of the Bio-Oss[®] particles (Fig. 4C), but this is not a general phenomenon 6 months after grafting. Although some scalloping of the surface of the Bio-Oss[®] particles was noted, no osteoclasts were found in the specimen. Thus, convincing evidence of resorption of the Bio-Oss[®] particles or signs of remodelling could not be demonstrated in our samples. Again, remnants of the Bio-Gide[®] membrane were not detected.

Histomorphometry

The mean TBV was in the Bio-Oss[®] group significantly lower when compared to areas grafted with chin bone ($p < 0.01$, t -test), but the mean TMM was comparable between the three groups. The MCTV in the Bio-Oss[®] group was comparable to the MCTV of the chin bone groups. The RBOV mounted for the remaining part (Table 2).

Discussion

The search for the ideal augmentation material still goes on. Although autologous bone is generally well accepted by most of the patients, autologous bone always involves donor site surgery and thus donor site morbidity^{18,31}. Also when mixing allograft material with autologous bone, as often advocated, there still might be a need of an extra donor site in case not sufficient bone can be harvested near the implant site³⁸. The results of this study indicate that also buccal grafting of a local defect in the aesthetic zone of the maxillary alveolar ridge with Bio-Oss[®] might provide a basis for reliable placement of implants.

The ideal biopsy for histological evaluation of the bone at the implant site

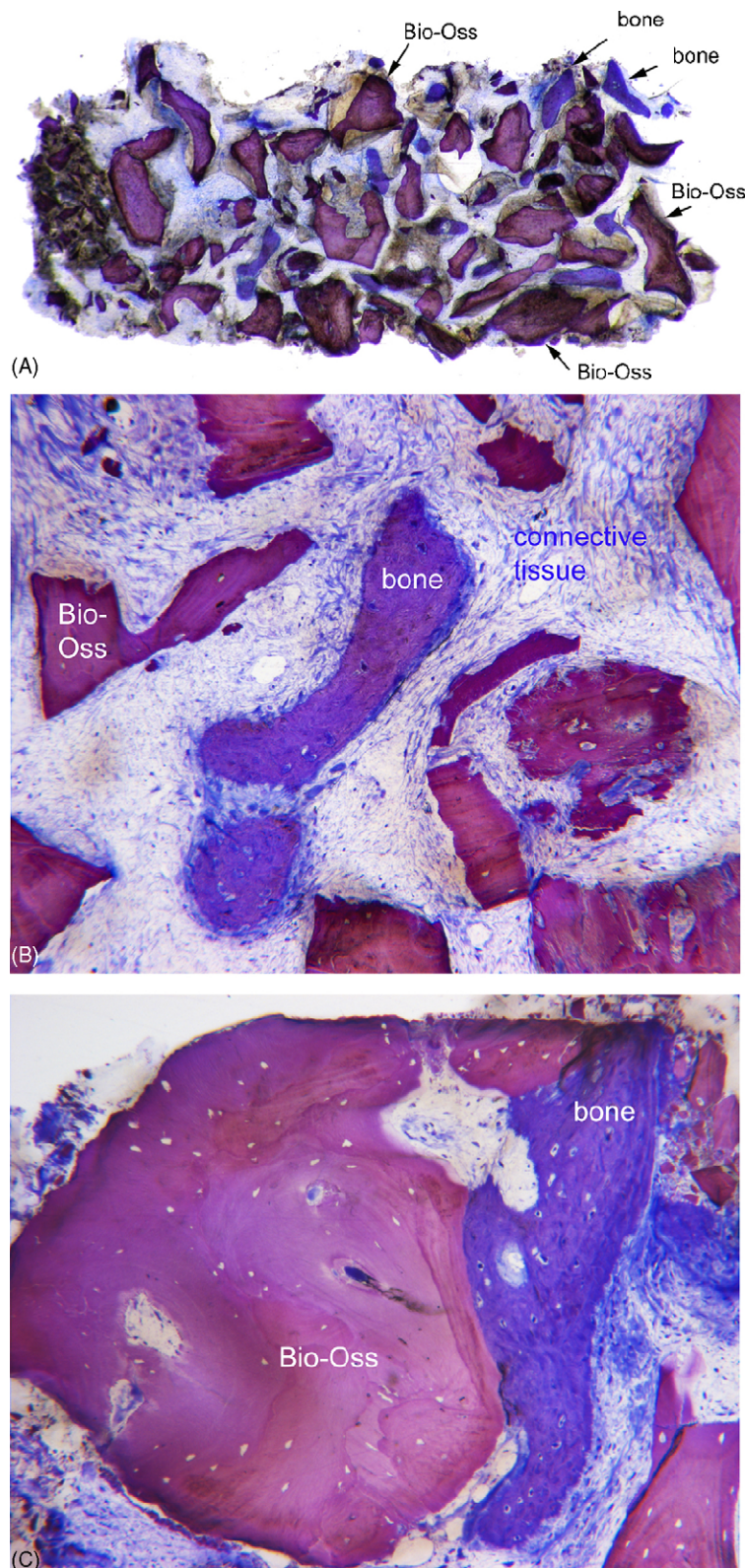


Fig. 4. (A) Section of a biopsy specimen after augmentation with Bio-Oss[®]. The Bio-Oss[®] particles are surrounded by connective tissue. Locally vital, newly formed bone is observed. (B) Detail showing the relation between Bio-Oss[®] particles, connective tissue and newly formed bone. (C) Detail showing newly formed bone in close contact with a Bio-Oss[®] particle suggesting apposition of bone on the surface of Bio-Oss[®] particles. The lacunae in the newly formed bone contain osteocytes.

should be taken from the actual implant site and has a diameter and length equal to the implant. It is possible, as shown in this study, to equal the actual implant length and the implant site, but by equalling the diameter of the implant-to-be-placed in the biopsy there is an inherent risk of adversely affecting the initial stability of the implant. For this reason we used a trephine with a diameter slightly less than the diameter of the implant. Even this method includes to a certain extent the deficits of methods harvesting biopsies next to the implant site or perpendicular to the long axis of the implant^{22,34,35} as in one of our biopsies no grafting material was detected.

The biopsy in which no grafting material (Bio-Oss[®]) was present most likely was taken from pre-existing autologous alveolar bone just palatal from the augmented area not touching the augmentation tissue. This might give rise to the assumption that in this patient augmentation of the defect was not necessary. It is still possible, however, that after taking the biopsy (using a 2.0 mm trephine bur) the augmentation material was exposed to the drill hole side after using the final drill for the implant surgery (diameter 3.8 mm). Alternatively, the applied histological processing technique (only the central part of the biopsy) might be responsible for the absence of augmentation material in the biopsy. But even if the implant is not in direct contact with the grafting material, it is thought that augmentation is necessary in such cases. Because of a good long-term prognosis, bone grafting is not only applied for good initial implant stability, but also to provide the buccal bone plate a minimum thickness of 2 mm and to shape the buccal aspect of the jaw. This is to try to prevent resorption of a thin buccal bone plate and to support the buccal gingiva for an optimal aesthetic result of the peri-implant soft tissues. Furthermore, this observation stresses the purpose of our study, viz. to investigate the quality of the bone as close to the actual implant site as possible. When drawing conclusions from biopsies taken, e.g. next to the implant site or perpendicular to the long axis of the implant^{22,34,35} there is an inherent risk of drawing these conclusions from a misconception because the bone quality at the actual implant site might be different from the bone in the surrounding area.

In all biopsies with Bio-Oss[®] granules present, these granules maintained their volume as well showed some osteoconductive properties. The granules were embedded in areas with vital bone, but,

Table 2. Total bone volume (TBV), marrow connective tissue volume (MCTV), the remaining Bio-Oss[®] volume (RBOV) and the total mineralised mass (TMM)

	Mean TBV (%)	Mean MCTV (%)	Mean RBOV (%)	Mean TMM (%)
Chin bone <i>without</i> GBR membrane	55.2 ± 6.8	44.8 ± 6.8	–	55.2 ± 6.8
Chin bone <i>with</i> GBR membrane	57.7 ± 11.5	42.3 ± 11.5	–	57.7 ± 11.5
Bio-Oss [®] with GBR membrane	17.6 ± 14.5	41.9 ± 13.1	40.5 ± 9.3	58.1 ± 13.8

as no convincing signs of resorption were observed, 6 months after grafting the Bio-Oss[®] material was still in place. On the surface of the Bio-Oss[®] particles some scalloping was observed, but presence of osteoclasts could not be shown. Therefore, it is not possible to state whether this scalloping is due to post-grafting osteoclastic activity. Also YILDIRIM *et al.*³⁸ found no osteoclastic activity after 6 months in humans. Other authors suggest a slow but predictable resorption of Bio-Oss[®] in humans^{1,12,34,35}. This probably will occur, but only on the long term as PIATELLI *et al.*²⁸ observed the presence of osteoclasts in the process of resorbing the Bio-Oss[®] particles and formation of neighbouring newly formed bone in biopsies retrieved after 18 months and 4 years. These human data are in contrast to animal data reporting that Bio-Oss[®] appears to be progressively resorbed during a 3–7-month period and became integrated and subsequently replaced by newly formed bone^{2,14,19,23}.

In all areas grafted with chin bone non-vital bone was still present 3 months after augmentation. This is in full agreement with the study of ZERBO *et al.*⁴⁰ reporting that non-vital bone is replaced by new vital bone in approximately 7 months.

Notwithstanding the fact that the healing time in the Bio-Oss[®] group was double of that of the chin bone groups, 6 months after augmentation the majority of the Bio-Oss[®] granules was not replaced by bone. The RBOV found in this study is comparable to the RBOV reported in the literature^{12,26,28,38,39}. Obviously, the Bio-Oss[®] group can be expected to render smaller values for the TBV than chin bone groups due to the fact that part of the defect space is filled with Bio-Oss[®] particles. When the RBOV is added to the calculation, similar values for the TMM were obtained for the Bio-Oss[®] group and the chin bone groups. Despite the relatively low osteoinductive capacity of symphyseal bone compared to other autologous bone grafts, it is still superior to Bio-Oss[®] considering the difference in healing time (3 months) needed to achieve the same results.

It was noted that the initial stability of the implants was good in all cases although the bone at the implant sites

reconstructed with Bio-Oss[®] clinically was not as compact as the bone at sites reconstructed with chin bone. A possible explanation for this is that in the Bio-Oss[®] cases the apical part of the implants could have been inserted in pre-existing alveolar bone, in which case the support of the pre-existing alveolar bone is responsible for the good initial stability of the implants rather than the Bio-Oss[®] augmentation material. As it takes more than 6 months for the implants to be loaded (abutment connection is performed 6 months after implant placement) further ingrowth of bone and suggested remodelling of the grafting material probably will have occurred thus allowing for loading of the implants.

From this study, it is concluded that at the time of placement of the implants the grafting material is still not fully replaced by new vital bone. In case of Bio-Oss[®], most of the grafting material is even still present. Despite these differences, the 1-year clinical results were very good and comparable between the various grafting techniques applied.

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